Natural Toxins in Plant Foodstuffs

Since food plants are mixtures of large numbers of chemical compounds and since any substance is toxic in a high enough dose, it is not surprising that natural food plants are toxic under certain conditions. Toxicity from common natural foods has resulted from long-term consumption of a single food item or from short periods of consumption of foods containing unusually high levels of a toxic substance. The process of diet selection has been continuous since prehistory and has minimized consumption of foods of high toxicity.

I. Natural Goitrogens

Human goiter remains a significant problem in certain parts of the world. Only about 4% of human goiter is thought to be due to causes other than iodine deficiency. The cause of endemic goiter may often be interactions of factors such as iodine deficiency and certain food components. In some areas of the world, dietary cruciferous plants could be one of the contributing factors.

Methods of determining the goitrogenic activity of a substance have improved greatly over the years. The earliest method involved visually inspecting and weighing the thyroid glands of experimental animals fed the test substance. More recently, histological examinations of the glands have been used to provide additional information about the nature of the agents or the conditions that cause enlargement. Other criteria used to assess goitrogenic activity are growth rate of the test animal, basal metabolic rates, and assays for iodine content of the thyroid and blood. Current tests consist of measuring the uptake of radioactive iodine by the thyroid gland following feeding of the test material. This procedure has been used with rats, chicks, and humans, and the anti-thyroid response to a substance has been shown to vary with species. Advantages of this test over previous tests are its increased speed and sensitivity. A disadvantage is that the test gives no information on the cumulative anti-thyroid effects of feeding low levels of goitrogen-containing natural products. Examination of this aspect of the problem requires extended administration of the test material, usually in feed of known iodine content, followed by an examination of the thyroid glands.

Goiter can be consistently induced in animals when the seeds of certain Brassica species are included in the feed. However, thyroid enlargement is variable and does not occur when the leafy portion of the vegetable is included in the feed. It is unlikely that consumption of Brassica plants as a normal part of an otherwise adequate diet will induce thyroid enlargement. However, it seems plausible that consumption of unusually large amounts of some of these plants (such as cabbage) might cause thyroid abnormalities. In particular, consumption of Brassica might have contributed to the relatively high incidence of goiter in areas of the world where the dietary iodine intake is low.

The goitrogenic substances in Cruciferae such as goitrin, of which family Brassica is a genus, are formed from glucosinolates (Figure 5.1). These substances may be converted to several products following the action of the
enzyme thioglucosidase, which is present in all plants that contain glucosinolates and in certain microorganisms, including gut bacteria. Products of this reaction include nitriles, thiocyanates, and oxazolidines.

The oxazolidine goitrin is a thyroid-suppressing substance as measured by reduced uptake of radioactive iodine and by thyroid enlargement in animals. The racemic mixture of R- and S-goitrin has biological activity equivalent to that of pure goitrin in either optically active form. The activity of goitrin is species-dependent and shows 133% of the activity in man of propylthiouracil, and anti-thyroid drug. Goitrin has relatively weak anti-thyroid activity in rats since long-term feeding of goitrin at 0.23% of the diet causes only mild thyroid enlargement.

Thiocyanate (SCN—) may occur in plants chiefly as a product of glucosinolate and isothiocyanate breakdown. Thyroid enlargement by thiocyanate results from inhibition of iodine uptake by the thyroid gland and is magnified with iodine deficiency.

The mixed nitrile fraction or modified Brassica meals known to contain a mixture of nitriles are of established toxicity to rats. The toxicities of these nitriles are increased apparently because of the presence of more reactive moieties such as the epithio— or allylic—alcohol components. Such structural components predispose these compounds to nucleophilic attack by key cellular substances, which may result in toxic effects.

Figure 5.1 Glucosinolate compounds and their formation pathways.

Goitrogenic levels of goitrin and thiocyanate are not likely to occur in cow’s milk. However, these substances in the feed of cows appear to reduce iodine uptake by the mammary gland, which results in production of milk low in iodine. Thus, milk low in iodine may contribute to goiter development in people heavily dependent on milk as a primary iodine source.

A. Mode of Toxic Action

Secretion of the thyroid hormones thyroxine and triiodothyronine is regulated by the hypothalamus and the pituitary gland in the following manner. The hypothalamus produces thyrotropin-releasing hormone (TRH) which stimulates the pituitary gland to synthesize and release the thyroid-stimulating hormone (TSH). TSH promotes the uptake of iodine by the thyroid, the synthesis of thyroglobulin, and the release of thyroxine and triiodothyronine. Increased concentrations of the thyroid hormones reduce TSH secretion by a negative feedback
mechanism. Alterations in the output of these thyroid hormones result in important changes in oxygen consumption, cardiovascular function, cholesterol metabolism, neuromuscular activity, and cerebral function. Growth and development are also seriously affected when the production of thyroid hormones is deficient. Triiodothyronine is four times as active metabolically as thyroxine. In humans, triiodothyronine is responsible for two-thirds of the biological activity of the thyroid hormones and thyroxine accounts for one-third of the activity.

Several steps are recognized in the synthesis of thyroid hormones. They include

1. concentration of inorganic iodide (iodine trapping);
2. oxidation of iodide to free iodine or hypoiodite;
3. formation of monoiodotyrosine and diiodotyrosine; and
4. coupling of two diiodotyrosines to form thyroxine (tetraiodothyronine).

Enzymes in the liver, kidney, and other organs remove one iodine atom from thyroxine and convert it to triiodothyronine. It is believed that more than half of all circulating triiodothyronine is made by peripheral deiodination of thyroxine and only about one-third is secreted by the thyroid gland.

Substances that depress thyroid function may be placed into one of several categories on the basis of their mode of action. Substances such as goitrin and thiourea inhibit thyroxine synthesis. Substances such as thiocyanates and nitrates inhibit iodide uptake by the thyroid gland through a mechanism that is poorly understood. Substances which inhibit thyroxine synthesis (i.e., goitrin) do not diminish the iodide-concentrating ability of the thyroid gland, but rather block formation of the iodinated amino acids. These substances inhibit thyroxine peroxidase, the iodide oxidizing enzymes. They block the reactions that require free iodine. When substances in this group of inhibitors of thyroxine synthesis are administered to humans or experimental animals, the performed thyroxine continues to be secreted. However, thyroxine secretion diminishes as the stored organic iodine becomes exhausted because of lack of resynthesis. This causes increased secretion of the TSH, which produces a hyperplastic, highly vascularized thyroid gland that has a greatly increased capacity for iodide trapping.

II. Cyanogenic Glycosides

Cyanogenic glycosides are a group of widely occurring natural substances that on hydrolysis yield a ketone or aldehyde, a sugar, and the highly toxic cyanide ion. Toxicity of cyanogenic glycosides is due to the liberation of cyanide (see Table 5.1 for the major food and feed sources of cyanide). Cyanide release from cyanogenic glycosides occurs readily in the laboratory by acid or base hydrolysis. However, hydrogen cyanide release is not appreciable in the stomach in spite of the decidedly acidic nature of its contents. Hydrogen cyanide is released from cyanogenic glycosides in chewed or chopped plants or following ingestion by an enzymatic process involving two enzymes (Figure 5.2). The first step is cleavage of the sugar, catalyzed by 13-glucosidase, which yields a cyanohydrin and a sugar. Most cyanohydrins are relatively unstable and spontaneously decompose to the corresponding ketone or aldehyde and hydrogen cyanide. However, this decomposition is accelerated by the action of the enzyme, hydroxynitrile lyase. The cyanogenic glycoside and the enzymes necessary for release of hydrogen cyanide are all present but separated in the plant. When fresh plant material is macerated as in chewing, cell structures are broken down sufficiently to allow the enzymes and the cyanogenic glycoside to come into contact to produce hydrogen cyanide. This is thought to be the principal mechanism of cyanide poisoning from consumption of fresh plant material.

Several methods, including chopping and grinding, have been developed to detoxify cyanogenic food products. In practice, cassava, which is an important source of carbohydrate for people in South America and Africa, is most often chopped and ground in running water, a process which can remove both cyanogenic glycosides and any released hydrogen cyanide. Fermentation and boiling processes are also used in the production of cassava flour. In spite of this well-developed processing procedure,
the cyanide content of cassava products can remain significant. In general, the more extensively purified cassava flours are the most expensive, which generally forces individuals with limited financial resources to depend on the more heavily contaminated flour as a food.

Purified cyanogenic glycosides or cyanogenic glycosides in food that has been boiled to inactivate enzymes produce somewhat variable toxic effects in animals and people. Purified cyanogenic glycosides, i.e., amygdalin, fed to guinea pigs in very large doses produced no toxic effect. Although cyanogenic glycosides are stable in saliva and gastric juices, consumption of twice-boiled lima beans known to contain cyanogenic glycosides produces symptoms of acute cyanide poisoning, and lima beans boiled for 2.5 hr induces vomiting and increased levels of urinary cyanide. This evidence indicates that people may harbor intestinal organisms that contain the enzymes necessary to free cyanide from ingested cyanogenic glycosides.

A. Cyanide Toxicity

Cyanide is considered a highly toxic substance. Symptoms of acute poisoning include mental confusion, muscular paralysis, and respiratory distress. The minimal lethal oral dose of hydrogen cyanide is estimated to be 0.5—3.5 mg/kg body weight. Cyanide exerts its toxic effects by binding to the ferric ion of cytochrome oxidase in mitochondria. The overall effect is cessation of cellular respiration.

The cyanide ion is normally metabolized as indicated in Figure 5.3. The principal excretion product of cyanide is thiocyanate, the production of which is catalyzed by rhodenase, an enzyme that is widely occurring in most mammalian tissues. Minor metabolic routes of cyanide involve reaction with cysteine to produce a thiazoline and an oxidative pathway leading ultimately to carbon dioxide and formaldehyde. An additional minor metabolic pathway for cyanide is complication with hydroxycobalamin. [his complication may be the normal metabolic route of small amounts]
cyanide in the body.

The usual treatment for acute cyanide poisoning is administration of nitrite or nitrite esters such as amyl nitrite, which converts hemoglobin Fe(II) to methemoglobin (Fe(III)). Increased circulating levels of methemoglobin will draw cyanide away from cytochrome oxidase, thus allowing cellular respiration to proceed. Final detoxification of the cyanide is facilitated by administration of thiosulfate required for formation of thiocyanate.

Although the effects of acute cyanide poisoning are fairly well-defined, the results of chronic cyanide poisoning are less well established. Consumption of cassava in certain parts of Africa and South America is associated with at least two disorders that do not seem to occur in areas where cassava consumption is low or in individuals who consume cassava free of cyanide. A disorder known as tropical ataxic neuropathy (TAN) and characterized by optic atrophy, ataxia, and mental disorder is found in areas of West Africa where cassava is a staple of the diet. Individuals with this disorder have very low concentrations of sulfur amino acids in the blood and elevated levels of plasma thiocyanate. Symptoms of the disease subside when patients are placed on cyanide—free diets and recur when traditional eating habits are resumed. Goiter is also prevalent in these areas. This is not surprising in view of the elevated blood levels of thiocyanate, an established goitrogen.

A related syndrome that is associated with prolonged consumption of cyanide is known as tropical amblyopia. This disease, characterized by atrophy of the optic nerve, resulting in blindness, is prevalent in populations consuming cassava as a staple in the diet. Long-term administration of sublethal doses of cyanide to animals results in destruction of optic nerve tissue. Similar effects have been seen in people exposed to low concentrations of cyanide for long periods of time.

The toxic effects of chronic cyanide consumption are modified by other dietary components, and cyanide-induced goiter is not observed if the diet has adequate levels of iodine. Cyanide-induced neurological destruction is generally seen only in partially malnourished populations. The ultimate source of sulfur required for conversion of cyanide to thiocyanate is sulfur-containing amino acids. Diets deficient in these substances result in a decreased ability to detoxify cyanide and increased circulating levels of cyanide. Chronic consumption of cyanide in marginally protein-deficient diets can magnify the sulfur deficiency of these diets. Thus, consumption of foods containing cyanogenic glycosides may not only result in toxic effect directly attributable to cyanide, but may indirectly promote effects characteristic of protein malnutrition.

![Figure 5.3 Normal metabolism of cyanide.](image_url)
III. Favism

Favism is a syndrome of acute hemolytic anemia induced by consumption of raw or cooked Vicia fava beans, commonly known as broad beans or fava beans. Favism is generally restricted to populations near the Mediterranean Sea or in China. The disease occurs to a greater extent in males than in females and is more severe in infants and young children than in adults. While adult deaths from favism rarely occur, fatalities have been reported in infants and children. The clinical symptoms of favism may include pallor, fatigue, shortness of breath, nausea, abdominal pain, fever, and chills. Renal failure occurs in the more severe cases. The onset of symptoms generally occurs within 24 hr following ingestion of the bean and persists for up to 2 days. Recovery in most individuals is spontaneous and abrupt.

Studies of the etiology of favism have been hampered by the unavailability of a suitable animal model for the disease. However, the results of several epidemiologic studies indicate that susceptible individuals have decreased levels of both glucose-6-phosphate-dehydrogenase (G6PD) and reduced glutathione (GSH) in red blood cells. G6PD catalyzes a reaction in glucose metabolism that produces NADPH. Adequate levels of GSH in turn are maintained by the glutathione reductase-mediated reaction of oxidized glutathione (GSSG) with NADPH. Thus, reduced levels of G6PD result in a diminished capacity of cells to maintain normal levels of GSH. Adequate levels of GSH, an antioxidant, are required to maintain stability of the cell membrane.

In experiments with suspensions of human red blood cells, it was found that GSH levels of cells from individuals susceptible to favism are affected by components of the fava bean included in the suspension mixture. GSH levels of cells from normal individuals do not show this sensitivity. The active substances in fava bean are pyrimidine derivatives, divicine, and isouramil, which are the corresponding aglycones of vicine and convicine (Figure 5.4). These aglycones are readily oxidized in air and rapidly promote the nonenzymatic conversion of GSH to GSSG in solution. Thus, it is suggested that these pyrimidine derivatives formed from the corresponding glycosides by enzymatic action in the plant or in the gut maybe causative agents of favism. Confirmation of this hypothesis must await development of a suitable animal model for the disease or appropriate tests in humans.

IV. Lathyrism

Lathyrism is an ancient disease caused by consumption of certain peas of the genus Lathyrus, known as vetch peas, chick-peas, or garbanzos. The disease is primarily restricted to areas of India where epidemics of the disease still occur. Although L. sativus is well known to be toxic and its cultivation and sale in most parts of India are banned, its hardiness under adverse growing conditions and its resistance to drought make it a soughtafter crop. Lathyrism has two manifestations, osteolathyrism and neurolathyrism.

Osteolathyrism is a disease seen in animals consuming various lathyrus species. The disease is characterized by bone deformations and weakness in aortic and connective tissue. Although many substances have been tested and found to have osteolathyrogenic activity, the lathyrogenic activity of lathyrus species seems to be restricted to a single substance, 13-L-glutamylaminopropionitrile (BAPN, Figure 5.5). When BAPN was included in the diet of rats at the level of 0.1—0.2%, skeletal deformity and aortic rupture developed.

The mode of action of BAPN in osteolathyrism has been studied in some detail. The principal effect of BAPN is to inhibit the cross-linking of collagen, the primary structural protein of connective tissue and bone. Collagen cross-linking requires an initial oxidative deamination of peptide bound lysine, catalyzed by the enzyme lysyl oxidase. The oxidized lysine residues combine with amino acids on adjacent peptide chains, forming the insoluble cross-linked collagen. BAPN irreversibly inhibits lysyl oxidase, thus preventing the formation of the collagen network.

Neurolathyrism is the form of the lathyrus-induced disease that affects humans. The disease, caused by long-term consumption (longer than 3 months) of L. sativus, is characterized by increasing paralysis of the legs, followed by general weakness and muscular rigidity. The onset of the symptoms is often sudden and may be initiated with a sudden contraction of the calf muscles of the leg. Most cases of the disease involve young men.
Studies of the etiology of neurolathyrism have been hampered in the past by the inability to reproduce this disease in laboratory animals. In initial studies, crude extracts, purified fractions from *L. sativus*, were tested for activity by injection into day-old chicks. Toxic reactions include convulsions and other reactions indicative of neurological damage. In these early studies, P-N-oxalyl-L-a,β-diaminopropionic acid (OPAP, Figure 5.6), which is absent in other species of *Lathyrus*, was isolated from *L. sativus*. ODAP produced neurological responses in young rats, young guinea pigs, and young dogs. However, neurological symptoms in adult rats could be seen only on injection of ODAP into the brain. Neurologic symptoms have been induced in adult squirrel monkeys by intraperitoneal injection, and selective concentration of ODAP in the cerebellum of these monkeys was noted. Thus, although a role of ODAP as the causative agent in human neurolathyrism has not been proven, the data accumulated in studies with animals support this hypothesis.

The mechanism by which ODAP exerts its toxic effect on the nervous system has not been established. However, there is increasing evidence that ODAP may interfere with the normal function of glutamic acid at the nerve synapse. ODAP competitively inhibits the uptake of glutamic acid into the cells of various microorganisms. ODAP also inhibits the uptake of glutamic acid by synaptic components of rat and monkey nervous systems. Release of glutamic acid from these synaptic components is enhanced, however, following treatment with ODAP. Thus, the overall effect of ODAP appears to be a net increase of glutamic acid concentration at the synapse. The significance of this increase in terms of the toxic lesions of neurolathyrism remains to be determined.
Lectins are a rather remarkable group of proteins and glycoproteins that possess the ability to bind certain carbohydrates. When these carbohydrates are components of cell walls, lectins will cause the agglutination of the cells which contain them. The ability of lectins to agglutinate red blood cells is used as a basis for assays of blood types. When lectins bind to carbohydrate components of intestinal epithelial cells, the result may be a decreased absorption of nutrients from the digestive tract.

Lectins are widely distributed in nature. Extracts from over 800 plant species and from numerous animal species show agglutinating activity. Of particular interest here are the lectins that occur in various legumes used as feed or food sources. Lectin activity has been shown to occur in a wide variety of legumes used for food such as black beans, soybeans, lima beans, kidney beans, peas, and lentils.

Although lectins are a group of substances which have been recognized because of their ability to agglutinate or clump red blood cells, some of these substances are also highly toxic to animals. For example, lectins isolated from black beans produce growth retardation when fed to rats at 0.5% of the diet, and lectin from kidney beans produces death in rats fed on lectin at 0.5% of the diet for 2 weeks. Soybean lectin, a less toxic lectin, fed at 1% of the diet to rats produces only growth retardation. The LD₅₀ of soybean lectin is estimated as 50 mg/kg. Ricin, a lectin from the castor bean, is one of the most toxic natural substances with an LD₅₀ by injection of 0.05 mg/kg. Because of their high toxicity, castor beans (not a legume) must be thoroughly heated to deactivate their ricin before they can be used as animal feed.

The exact role of lectins in the anti-nutritional or toxic effects of various beans and legumes is the subject of some controversy and appears to depend on the specific legume in question. Uncooked beans as a major component of the diet generally do not support the growth of animals. Thoroughly heated beans, of course, do support growth. When the lectin fractions of black beans and kidney beans are fed to animals along with the heated bean material, toxic symptoms are manifest. In the case of soybeans, about half of the growth depression caused by raw soy meal can be attributed to the lectin. In addition, little improvement in nutritional quality is observed for soybean meal from which the lectin component has been removed. Thus, in addition to lectins, other substances such as inhibitors of digestive enzymes appear to contribute to the growth-depressing effects of raw beans.

The mechanism by which lectins produce an ultimate toxic effect is also open to controversy. It is well established that lectins from various sources present on the intestinal epithelium adsorb nutrients and thus reduce the absorption of those nutrients by the intestine. The resulting inefficient use of nutrients may in itself account for the poor growth promoted by diets rich in uncooked legumes. This effect may also magnify the protein losses induced by pancreatic hypersecretion caused by trypsin inhibitors also present in the legumes (see following). However, the microflora of the gut also appear to play a role in legume- and lectin-induced toxicity. Germ-free birds (i.e., birds free of intestinal bacteria) used as test species show less growth depression when fed raw legumes or isolated lectins than do conventional birds. For example, diets containing raw jack beans meal produce high mortality in Japanese quail. However, germfree birds exhibit no toxic effects under exactly the same experimental conditions; these observations have led some investigators to suggest that the lectins may impair the body’s defense system against bacterial infection, resulting in an increased tendency for an invasion by gut and other bacterial flora.
VI. Pyrrolizidine Alkaloids

The pyrrolizidine alkaloids (Figure 5.7) are a group of substances of related structure produced by a variety of plants, including many range plants (*Senecio*, *Crotalaria*, *Heliotropium*) that are consumed by livestock. Over 100 pyrrolizidine alkaloids have been isolated from various plants, and the levels range from traces up to 5% of the dry weight of the plant. Some of the compounds are potent carcinogens. Administration of one of the plants, *Senecio longilobus*, at 0.5% of the diet every other week induced tumors in 17 of 47 rats that survived the test. The doses of pure compounds required to induce carcinogenesis are moderately high, however. In one experiment, the pyrrolizidine alkaloid, monocrotaline, was given intragastrically to rats once a week at a dose of 25 mg/kg for 4 weeks, then at 7 mg/kg body weight for 38 weeks. This regimen induced cancers in approximately 25% of the animals treated. In another experiment, weekly intraperitoneal injections of lasiocarpine at a dose of 7.8 mg/kg body weight for 1 year produced no tumors. However, following this year of treatment, a high percentage of the survivors developed malignant tumors of the skin, bones, liver, and other organs.

It is not certain whether these substances are passed along to humans in products such as milk and meat. Some of the pyrrolizidine-containing plants are used in herbal remedies and for tea preparations. In addition, one species of comfrey, known to contain pyrrolizidine alkaloids, is used as a green vegetable in Japan. However, the importance of pyrrolizidine alkaloids in human carcinogenesis is as yet unclear.

The carcinogenic and mutagenic activity of pyrrolizidine alkaloids is dependent on metabolism to an ultimate, reactive form. The presence of the 1,2-double bond in the pyrrolizidine nucleus appears to be required for carcinogenic activity. The exact role of this double bond has not been determined with certainty, although epoxidation at this site is a likely possibility. The resulting epoxide is then subject to nucleophilic attack. However, the 1,2-double bond also facilitates dehydrogenation to the corresponding pyrrole, which may also be subject to nucleophilic attack.

VII. Enzyme Inhibitors

Detection of biological activity is, of course, dependent on the development of an appropriate bioassay. For reasons of sensitivity, convenience, and cost, most bioassays incorporate some *in vitro* techniques. It is important to remember, however, that this *in vitro* activity may not be relevant to biological effects *in vivo*. While it is true that the hemagglutinating activity of the lectins discussed in a previous section has been used in blood type determinations, this activity is of little importance to the toxicity of some of these substances *in vivo*. Another readily determined property of foods or their components is their ability to inhibit certain enzyme-mediated reactions. Bioassays for this type of activity generally require incubation of a specific enzyme and its substrate along with the substance to be tested. The importance of enzyme activity to the potential toxicity of a food or food component is not always clear. In this section protease inhibitors and cholinesterase inhibitors will be discussed.
A. **Protease Inhibitors**

Inhibitors of enzymes involved in the hydrolysis of protein (protease inhibitors) are widespread throughout the plant kingdom. Legumes are a major source of these substances, although they also occur in other foods. Inhibitors of trypsin, a gastric protease, have been isolated from most varieties of legumes and grains as well as from other foods such as potatoes, eggplant, and onions.

Indications that trypsin inhibitors play a role in the anti-nutritional properties of certain legumes come from animal experiments. The growth of several species of laboratory animals is inhibited by anti-tryptic components from beans. Addition of purified trypsin inhibitors to diets containing predigested protein or amino acids causes an obvious growth retardation in rodents. However, the protease-inhibitory effect of these substances does not appear to be the cause of the decreased growth rate. In addition to the growth-retardant effect of the trypsin inhibitors, pancreatic hypertrophy is observed in some animal species. The attendant hypersecretion of pancreatic enzymes combined with the marginal levels of certain essential amino acids in soy protein are a possible cause of the anti-nutritional effects of raw beans. Selective removal of the trypsin inhibitor results in a 40% decrease in the pancreatic hypertrophic effect of the original raw beans. In addition, supplementation of raw soy meal with certain amino acids eliminates the growth-depressing effect of raw meal while not affecting the pancreatic hypertrophic effect. Thus, it appears that the pancreatic hypersecretion of proteins rich in amino acids that are marginal in the diet results in amino acid deficiency and attendant growth depression.

B. **Cholinesterase Inhibitors**

Cholinesterase is an enzyme that mediates hydrolysis of acetylcholine to acetate and choline. Acetylcholine, present in vesicles in the axonal terminus, is the substance responsible for transmission of the nerve impulse across the synapse. Stimulation of the vesicles causes the release of acetylcholine, which diffuses across the synapse and initiates the impulse in the adjacent neuron. Once the nerve impulse is transmitted, the acetylcholine must be hydrolyzed so that the neuron can be repolarized in preparation for the next impulse.

Some plants contain compounds that inhibit cholinesterase activity, of which undoubtedly the most notorious plant is the West African calibar bean. This bean is the source of physostigmine (Figure 5.8), a potent cholinesterase inhibitor and a model for the carbamate class of insecticides. Preparations of this highly toxic and inedible bean have been used as an ordeal poison in witchcraft trials in Africa.

The anticholinesterase substance found in food products that has perhaps been studied the most is solanine (Figure 5.9), a glycosidal alkaloid (glycoalkaloid) composed of a carbohydrate residue and the aglycone, solanidine. Solanine is found primarily in members of the genus *Solanum*, which includes eggplant, potato, and tomato.

The total glycoalkaloid content of potato tubers varies with the variety and appears to be within the range of 20 to 100 mg/kg of fresh tissue. However, a variety (Lenape) developed for potato chips had a total glycoalkaloid content of about 300 mg/kg fresh tuber. The use of Lenape as a food product was discontinued. Glycoalkaloid levels of over 200 mg/kg fresh weight are now considered excessive and potentially dangerous. The FDA prohibits the sale of food products containing these levels.

Although solanine is found throughout the potato tuber, the greatest concentrations occur in the sprouts,
peelings, and sun-greened areas. In the sprouts, solanine represents about 40% of the total glycoalkaloids, with another similar compound, chaconine, comprising 60%. Chaconine differs from solanine only in the composition of the carbohydrate moiety.

Greening of the potatoes, whether by natural or artificial light, can considerably increase the levels of glycoalkaloids. For example, 5 days of exposure to white fluorescent light will increase the total glycoalkaloid content in the peel of a Russet Burbank variety from approximately 250 to 700 mg/kg. The green appearance of these potatoes is due to increased chlorophyll content which in itself is not hazardous.

Consumption of potatoes by people or animals occasionally has been reported to cause illness or death. Poisoning has resulted from ingestion of potato sprouts, sprouted potatoes, and greened potatoes. In one instance of human poisoning involving six people, the symptoms were described as increasing gastric pain followed by nausea and vomiting.

Respiration was difficult and accelerated with market weakness and prostration. In this instance, two people died approximately 1 week after consumption of the greened potatoes. Experimental solanine poisoning induced in human volunteers yields symptoms similar to those reported in the case of green potato poisoning. Doses of approximately 3 mg/kg caused drowsiness, itchiness in the neck region, increased sensitivity (hyperesthesia), and labored breathing. Higher doses caused vomiting and diarrhea. Gastrointestinal symptoms (abdominal pain, nausea, vomiting, and diarrhea) are also observed in human intoxication by organophosphate pesticides, which are potent cholinesterase inhibitors.

Although the symptoms of green-potato poisoning and those of acute solanine toxicity are quite similar and therefore implicate solanine as a causative factor, the level of solanine present in the toxic potatoes apparently is not high enough to produce the toxic symptoms by itself. Total alkaloid content of approximately 420 mg/kg of fresh potato has been determined in two cases of human poisoning from potatoes. With the assumption that total alkaloid is composed of 50% solanine, an individual would be required to consume as much as 1 kg of whole potato in order to approach the 200 mg of solanine determined to induce the initial signs of solanine toxicity. In addition, results of experiments with animals have shown that solanine is a substance of low oral toxicity. Thus, oral LD₅₀’s in sheep, rats, and mice are on the order of 500, 600, and over 1000 mg/kg, respectively. It appears, therefore, that solanine is but one of the causative agents of green-potato poisoning and probably acts in combination with other substances such as chaconine or other possibly minor components of potato. The toxicology of chaconine and other potato components requires further investigation.

![Figure 5.9 Structures of solanidine and related compounds.](image)
VIII. Vasoactive Amines

A wide variety of foods from plant and animal sources contain biologically active amines. Substances such as putrescine and cadaverine (Figure 5.10) may occur in meat and fish products as a result of bacterial action on certain amino acids. Other substances, such as dopamine and tyramine, may occur as natural components of certain food plants such as banana and avocado.

Substances that affect the vascular system are known as vasoactive amines. Vasoactive amines that constrict blood vessels and thereby increase blood pressure are known as pressor amines. The pressor amines, norepinephrine and dopamine (the catecholamines) (Figure 5.10), are important neurotransmitters released from adrenergic nerve cells. One of the many striking biological effects of intravenous administration of

![Figure 5.10 Structures of vasoactive amines.](image)

catecholamines in animals is a sharp rise in blood pressure due primarily to direct vasoconstrictive action of these substances. Tyramine, which is not normally a product of mammalian metabolism, can increase blood pressure by an indirect mechanism. Administered tyramine is taken up by the reabsorption process that normally controls intraneuronal levels of catecholamines. This reabsorption of tyramine displaces catecholamines from storage granules, thereby freeing catecholamines with an attendant rise in blood pressure.

Circulating levels of exogenous pressor amines and other vasoactive amines are carefully controlled by the action of monoamine oxidase (MAO), an enzyme of wide distribution in the body. Because of rapid metabolic conversion of amines by MAO and other enzymes, administration of pressor amines to normal mammals generally has little effect on blood pressure. However, marked pressor effects are observed when MAO is inhibited. Certain MAO inhibitors have been used in the clinical treatment of psychiatric depression. Examples of such drugs include isocarboxazid, nialamide, phenylzine sulfate, and tranylcypromine. One of the serious disadvantages of the clinical use of MAO inhibitors is the increased likelihood of adverse reactions to ingested foods that may release monoamines in the body. The ingestion of aged cheese, beer, or certain wines has caused severe hypertensive reactions in patients who were being treated with MAO inhibitors. A listing of amines in certain foods is indicated in Table 5.2. Observed symptoms included hypertensive crisis, migraine headaches, and in some cases, intracranial bleeding leading to death. The cause of these reactions was traced to the presence of tryamine in foods and beverages.
TABLE 5.2
Amine Content of Food Products (in µg/g)

<table>
<thead>
<tr>
<th>Food product</th>
<th>Serotonin</th>
<th>Tyramine</th>
<th>Dopamine</th>
<th>Norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana pulp</td>
<td>28</td>
<td>7</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Tomato</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Avocado</td>
<td>10</td>
<td>23</td>
<td>4—5</td>
<td>0</td>
</tr>
<tr>
<td>Potato</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0.1—0.2</td>
</tr>
<tr>
<td>Spinach</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Orange</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>—</td>
<td>120—1500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Camembert cheese</td>
<td>—</td>
<td>20—2000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stilton blue cheese</td>
<td>—</td>
<td>466—2170</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>—</td>
<td>26—50</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

IX. Mutagens in Natural Plants

A. Flavonoids

The flavonoids are a group of widely occurring plant substances used to flavor and color foods. One of these substances, quercetin, is the most common flavonoid compound in vascular plants (Figure 5.11). It occurs in conjugated or free form in many plant products such as fruits, vegetables, and teas. Quercetin and its close relative, kaempferol, tested mutagenic in the Ames assay. Quercetin is mutagenic without metabolic activation, but its activity is increased with the incorporation of the liver homogenate into the test system.

Other flavonoids, such as rutin, in which the 3-hydroxyl group of quercetin is conjugated with a carbohydrate, are not mutagenic unless an enzymatic preparation is included in the mixture that hydrolyzes the glycoside linkage. Such an enzymatic mixture is present in the intestines of humans and animals. Long-term feeding studies with quercetin have failed to show that the substance is a carcinogen. On the contrary, quercetin has anticancer properties.

B. Maltoles

Maltol, ethyl maltol (Figure 5.12), and diacetyl are weak mutagens. However, relatively large amounts of the substances are present in the diet. The usual levels of maltol added to baked goods, ice creams, and candy are approximately 110 ppm. Levels on the order of 80 ppm are added to certain beverages. Ethyl maltol, a more potent flavor enhancer than maltol, is generally used in concentrations of about 20 ppm in these foods.

In the United States, the average daily intake of maltol and ethyl maltol from all food categories for individuals 2—65 years old is estimated to be 29 and 5 mg, respectively. In certain individuals, the actual levels of consumption may be several times these averages. However, there is no evidence of ill effects in humans from normal dietary consumption of these substances. Results of experiments with dogs indicate that maltol and ethyl maltol are rapidly and efficiently absorbed following oral administration and converted to the glucuronide conjugate. Similar processes probably occur in humans.
Maltol, ethyl maltol, and diacetyl are representatives of the 1,2-dicarbonyl class of chemicals. The total daily human doses of mutagenic 1,2-dicarbonyl compounds are likely to be much greater than estimates based on known levels of maltol, ethyl maltol, and diacetyl. Other 1,2-dicarbonyl compounds such as intermediates in enzymatic and nonenzymatic browning reactions in foods are weakly mutagenic in the Ames test. No conclusive evidence showing the carcinogenic activities of these substances has been presented.

C. Caffeine

Caffeine is a methylated xanthine derivative (Figure 5.13) that occurs naturally in coffee, tea, cola, and cocoa products. The levels of caffeine in coffee range from 75 to 155 mg per 5-oz cup, with an average of about 115 mg. Traditional teas contain around 40 mg caffeine/cup, and milk chocolate and baking chocolate contain around 6 and 35 mg/oz, respectively. Caffeine is rapidly absorbed from the gastrointestinal tract and distributed throughout the body. It is metabolized and cleared from the bloodstream within a few hours in most people. However, clearance rates during pregnancy and in infants are considerably reduced. Caffeine causes a host of biological effects. At low doses of around 200 mg for an adult, caffeine produces (among other effects) central nervous system stimulation, diuresis, relaxation of smooth muscles, cardiac muscle stimulation, and increased gastric secretion. The centuries-old belief that caffeine improves physical performance in fatigued individuals has been substantiated scientifically, but performance of rested individuals is not affected by caffeine. Excessive consumption of caffeine can result in nervousness, irritability, and cardiac arrhythmias. The LD$_{50}$ for caffeine is estimated to be 200 mg/kg body weight, which puts caffeine in the moderately toxic range. Under laboratory conditions, caffeine causes many other effects, including teratogenesis, mutagenesis, carcino-
genesis, and anticarcinogenesis. Although none of these effects has been substantiated in humans, a panel of the United States National Academy of Sciences has recommended moderation in caffeine consumption for pregnant women.

D. Constituents of Spices

Spices are a prized group of minor components in human diets. The spice trade is one of the oldest trades known and the overland trade routes across the Old World predate recorded history. Spices include a variety of plant products, many with pungent flavors, that are used to enhance the natural flavors and aromas of foods and beverages. Spices often contain substances with potent biological activities. Examples of a few of these substances are discussed in the following section.

1. Onion and Garlic

Human consumption of onion (50—60 g), along with a high-fat diet, prevented both the increased tendency of blood to clot and the rise in serum cholesterol normally seen following the consumption of high-fat diets. Onion and garlic juice and the ether-extractable essential oils of these products have similar effects. Long-term feeding (4 months) of the essential oils of onion and garlic to rabbits decreased cholesterol-induced atherosclerotic lesions of the aorta by about one-half. The striking properties of onion and garlic essential oils have led some investigators to suggest the use of these products in the treatment of individuals who are predisposed to atherosclerosis and thrombosis. Components of these essential oils also show promise as anticancer agents.

2. Celery Oil

Two uses of celery seed oil in folk medicine are as a sedative and as a nerve tonic. Much of the aroma of celery seed oil can be ascribed to the presence of certain phthalides (Figure 5.14), one of which is called sedanolide. Although the name of this compound implies sedative activity, no such studies were actually carried out until fairly recently. Although sedanolide does not appear to be a generally occurring component of celery essential oil, other phthalides (3-n-butylnaphthalide and sedanenolide) are primarily responsible for the odor of this spice. Both of these substances are weak sedatives in mice. Because of the weakness of this activity, however, it appears that consumption of unusually large amounts of celery oil would be required to produce a sedative effect in people.

Figure 5.14 Structures of phthalides.
3. Licorice

Glycyrrhizic acid (Figure 5.15) is about 5—10% of the weight of the root of the licorice plant (*Glycyrrhiza glabra* L.). Consumption of large amounts of licorice candy (100 g/day) over an extended period has led to severe hypertension, sodium retention, and heart enlargement in people. These symptoms apparently have as their basis a corticosterone-like activity in which sodium and water are retained and potassium is depleted. Severe losses of potassium resulting eventually in extreme weakness and ventricular fibrillation were reported in a woman who habitually ate nearly 2 kg of licorice candy per week.

4. Nutmeg

Nutmeg and its close relative, mace, have been used extensively in folk medicine for a wide range of ailments, including digestive disorders, rheumatism, cholera, and flatulence. There have also been a number of reports of nutmeg poisoning due to its use as an intoxicant. Nutmeg apparently acts as an intoxicant through its depressor effect on the central nervous system. Reactions to nutmeg vary from no effects to full-blown hallucinogenic experiences like those caused by hashish or LSD. Distortions of time and space with feelings of unreality have been reported. Effects of a single dose of approximately 20 g of whole nutmeg are reported to subside within 12—48 hr. Continued use of moderate doses of nutmeg can result in liver damage and death. Side effects of even moderate doses of nutmeg include headache, cramps, and nausea. An active ingredient of nutmeg appears to be myristicin, which comprises approximately 4% of the oil. Myristicin (Figure 5.16) has also been identified in black pepper, parsley, celery, dill, and members of the carrot family. Pure myristicin is not as potent as whole nutmeg. Thus, it appears that other substances in addition to myristicin may be responsible for the psychoactive properties of this spice.

![Figure 5.15 Structures of glycyrrhetinic acid and glycyrrhizic acid.](image)

5. Sassafras

The essential oil of the root bark of the sassafras tree (*Sassafras albidum*) was used until 1960 in the United States as a flavor component of root beer. Teas prepared from the root bark are still apparently popular as tonics and for a variety of remedies in folk medicine. Results of a series of studies by the FDA showed that safrole, which comprises about 80% of the oil of sassafras, is a hepatocarcinogen in rats and mice. Administration of 0.04—1.0% of safrole in the diet of male and female rats for 150 days to 2 years produced hepatic cancers. As a result of these
findings, safrole is no longer allowed as a food additive in the United States. The FDA also revised the banned substances listing of safrole specifically to ban sassafras bark, which is used primarily in the preparation of sassafras tea. Safrole is a component of many essential oils such as star anise and camphor oil. It also occurs in smaller quantities in mace, nutmeg, Japanese wild ginger, California bay laurel, and cinnamon leaf oil.

Safrole is related chemically to other substances found in spices. For example, β-asarone is a principal component of calamus oil (derived from the roots of *Acorus calamus*). The amount of β-asarone in the oil depends on the variety of plant. The oil was formerly used in the preparation of vermouth and other flavored wines; however, β-asarone is no longer used legally in the United States because it was found to cause malignant tumors in the small intestine of rats fed on high doses. A similar substance is estragole, which is a component of tarragon oil, produced from *Artemisia dracunculus* and used as a flavor. Estragole causes liver cancer in young male mice.

Safrole provides an example of certain substances that are metabolically converted to the active carcinogenic forms. An extensive series of studies by Elizabeth and James Miller and their co-workers at the University of Wisconsin have demonstrated that safrole is metabolized in the rat and mouse to the corresponding benzylic alcohol (the proximate carcinogen), which, in turn, may be activated to the acetate or sulfate, the ultimate carcinogens (Figure 5.17). Nucleophilic attack on the double bond of the ultimate carcinogen by DNA may result in a heritable change in genetic material (a mutation). Subsequent expression of this altered genome may produce cancer. Because of the chemical similarities of safrole, estragole, and β-asarone, it is likely that they are activated by similar processes.
E. Phytoalexins

Phytoalexins are antibiotics produced by a plant in response to environmental stresses. Various invading organisms such as bacteria, viruses, fungi, and nematodes will induce the production of phytoalexins in plants. In addition, exposure to cold, ultraviolet light, physical damage, and certain chemical compounds such as metal salts, polyamines, and certain pesticides can elicit the production of phytoalexins. Because phytoalexins are produced in response to such a broad range of agents that are potentially toxic to the plant, they are called stress metabolites. The classic example of phytoalexin production occurs in potatoes inoculated with the blight fungus, Phytophthora infestans. When inoculated into the potato, certain strains of this fungus will initially grow rapidly, followed by a gradual slowing of growth. If an extract of the infected material is placed in contact with a pure culture of the same fungus, the fungus will not grow. This phenomenon has been observed in many other plants such as peas, green beans, broad beans, soybeans, carrots, and sugar beets in response to infection by fungi. It appears that certain polysaccharide components of the cell wall of many fungi elicit this response.

The chemical composition of phytoalexins in general indicates that they are produced by modification of the plant’s normal metabolism. Certain representatives of the widely occurring isoflavonoid and terpene classes of natural products are often responsible for the phytoalexin activity of injured plants (Figures 5.18, 19, and 20). Quantities of phytoalexin produced by a plant can be quite significant. For example, soybeans infected with the fungus *Phytophthora megasperma* produce a phytoalexin known as glyceolin, which can accumulate over a period of days from undetectable levels to more than 10% of the dry weight of the infected tissue.

In general, the toxicological aspects of phytoalexins have received little attention, but phytoalexins from partially rotted sweet potatoes have been studied in some detail. Consumption of sweet potatoes has been known to produce severe respiratory distress, pulmonary edema, congestion, and death in cattle. The sweet potatoes involved contained several toxic terpene substances (Figure 5.21). Two of the compounds, ipomeamarone and ipomeamaronol, cause liver degeneration in experimental animals (LD$_{50}$ 230 mg/kg). Lung edema factors have also been isolated from the infected sweet potato tuber. The substances (Figure 5.22), known as 4-ipomeanol (LD$_{50}$ 38 mg/kg), 1-ipomeanol (LD$_{50}$ 79 mg/kg), ipomeanine (LD$_{50}$ 26 mg/kg), and 1,4-ipomeadiol (LD$_{50}$ 104 mg/kg), all produce an acute toxic

![Figure 5.18 Structure of betavalugarin found in beets.](image)

![Figure 5.19 Toxic substances found in potatoes.](image)
response in mice that is indistinguishable from the acute response produced by the administration of the crude sweet potato extract.

Figure 5.20 Structures of glyceolin found in soybeans.

These toxic terpenes can occur in only slightly damaged sweet potatoes used as human food. The presence of these substances is always associated with darkening of the sweet potatoes. Ipomeamarone was shown in one study to be present in commercially available sweet potatoes at levels ranging from 0.1 to 7.8 mg/g sweet potato. Conflicting reports have appeared concerning stability of these toxic terpenes under normal cooking conditions. However, it appears that under conditions commonly employed in microwave cooking or baking, the concentration of ipomeamarone in sweet potatoes is reduced by 80—90%.

The phenomenon of toxic phytoalexin production must be considered in the debate over the use of pesticides. While pesticides have been used effectively to increase crop yield, their use has many drawbacks, including the nonspecific nature of their toxicity and their persistence in the environment. Plant breeders have been working, with some success,

Figure 5.21 Structures of ipomeamarone and its alcohol.

Figure 5.22 Toxic substances found in sweet potatoes.

to produce varieties of plants that are less dependent on pesticide use. However, this increased resistance of the various crops may result from high levels of phytoalexins. Until other means of pest control have been developed, it appears that the use of well-designed pesticides whose chemical, environmental, and toxic properties are already known may be preferable to developing new varieties of resistant crops with phytoalexins of unknown nature.